PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 27/447	A1	(11) International Publication Number: WO 00/16084 (43) International Publication Date: 23 March 2000 (23.03.00)

(21) International Application Number: PCT/GB99/03053

(22) International Filing Date: 15 September 1999 (15.09.99)

(30) Priority Data:
9819950.8 15 September 1998 (15.09.98) GB

(71) Applicant (*for all designated States except US*): UNIVERSITY OF SOUTHAMPTON [GB/GB]; Highfield, Southampton SO17 1BJ (GB).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): DAY, Ian, Nicholas, Monsarratt [GB/GB]; 6 Victory Way, Rownhams, Southampton SO16 8JZ (GB).

(74) Agents: HOWARD, Paul, Nicholas et al.; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

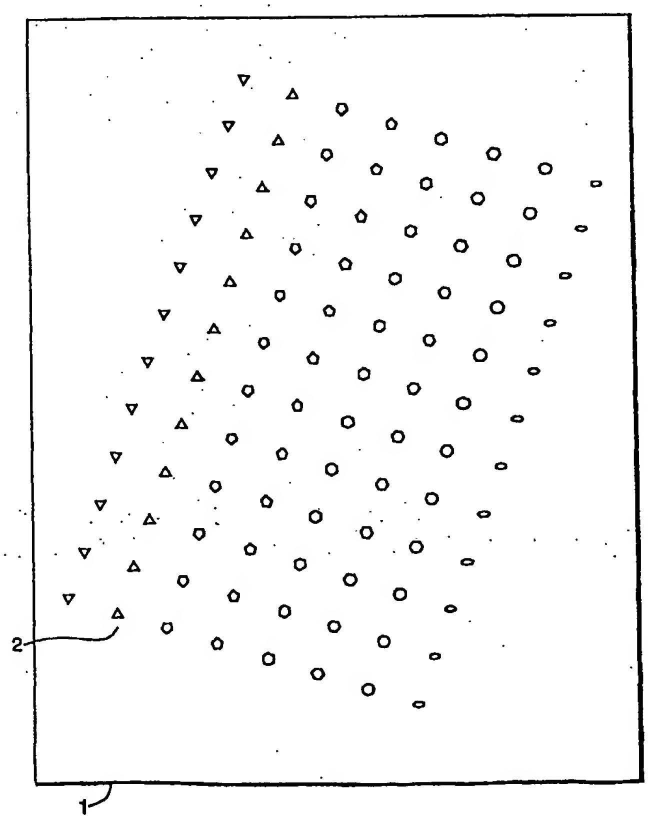
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ELECTROPHORESIS GEL-MATRIX

(57) Abstract

The present invention relates to an electophoresis gel-matrix layer comprising one or more wells having a front face which is curved and/or comprises more than one planar surface. The present invention also relates to methods for manufacturing the gel-matrix layers, uses of the gel-matrix layers and also kits for making the gel-matrix layers.



ELECTROPHORESIS GEL-MATRIX

The present invention relates to an electrophoresis gel-matrix layer having one or more wells formed in the gel-matrix layer for receiving a sample. The present invention also relates to a method of making the gel-matrix layer, uses of the gel-matrix layer and a kit for making the gel-matrix layer.

Electrophoresis of charged molecules, for example, DNA and RNA fragments using a gel-matrix of, for example, agarose or acrylamide is well known to those skilled in the art.

An agarose gel is usually formed by pouring the molten gel onto a horizontal plate. A well-former (comb) having a row of protruding teeth or pegs is positioned in the molten agarose while it sets. Once the agarose gel has set, the comb is removed to leave a series of wells in the agarose gel. The wells can then be loaded with samples which are then subjected to electrophoresis by applying a voltage across the agarose gel-matrix layer leading to the formation of electrophoresis tracks extending from each well parallel to the line of electrophoresis. The line of electrophoresis is defined as the line between the cathode and anode used to form the electrophoresis voltage across the gel-matrix layer.

In the case of an acrylamide gel it is necessary to sandwich the gel layer between two plates as acrylamide does not polymerise in the presence of air. Wells are formed in one end edge of the acrylamide layer by using a well-former (comb). Electrophoresis is carried out with the acrylamide gel sandwiched between the two plates in a vertical position with the wells formed in the upper edge of the gel.

The wells that are formed in such electrophoresis gel-matrix layers are generally rectangular or cube shaped.

US patent US-A-5,800,691 discloses rectangular shaped wells wherein the wells have an enlarged loading area at the open end of the well. US patent US-A-5,843,295 discloses a comb for forming rectangular shaped wells wherein the comb can also be used to guide sample delivery into the wells. US patent US-A-5,073,246 discloses wells which have a trough extending to one side of the well so as to increase the loading capacity of the well.

International patent application WO 96/18891 discloses electrophoresis gel layers having wells formed by square pegs so that the wells are substantially cube shaped.

All these prior art electrophoresis gel-matrix layers have wells wherein the front face of the well, namely the face through which the sample passes into the gel-matrix when an electrophoresis voltage is applied, is a flat planar face.

By having a flat planar front face, bands of the molecules in the sample migrate through the gel-matrix layer as discrete linear bands.

As indicated above, traditional approaches to electrophoresis employ rows of wells formed using a well-former (comb) either placed at the top of a gel formed vertically between two plates, or hanging vertically in a gel such as an agarose gel poured in a horizontal gel former. Generally, the well-former is a comb cut from a flat thin sheet of plastic or similar material. There is little scope for varying the dimensions (length) of a given well between cathode and anode relative to its width perpendicular to the line of electrophoresis.

A two-dimensional array of wells formed using a well-former (comb) which has its well-formers (teeth) arranged in two dimensions across the large flat surface of the overall comb has previously been described in International patent application WO 96/18891. Cutting the well-former has to be computerised machining rather than the simple "fretsaw" approach possible for traditional combs. International patent application WO 96/18891 describes a format using an 8 x 12 array of wells with a 9mm pitch, turned through a diagonal to lengthen the resultant gel track length from any well. Such a format is convenient because it is microplate compatible and also because air can be excluded by forming the gel adherent to one glass plate with a plastic well-former directly facing that plate. This enables the use of polyacrylamide, agarose and related matrices, and gives rise to open-faced horizontal submersible or semi-dry gels compatible with high throughput screening and with no sample re-coding.

Contrary to that disclosed in the prior art, it has been found that the use of wells having a non-planar front wall provides a number of advantages over the prior art electrophoresis gel-matrix layers.

The present invention provides an electrophoresis gel-matrix layer having one or more wells for receiving a sample formed in the layer, wherein the front face of the well is curved and/or comprises more than one planar surface.

The electrophoresis gel-matrix layer can be any gel-matrix layer suitable for use in electrophoresis, including agarose and agarose derivative gel-matrix layers, acrylamide and acrylamide derivative gel-matrix layers, and any physical, chemical or biological gel-

matrix layer. Preferred electrophoresis gel-matrix layers include agarose and acrylamide gel-matrix layers.

Preferably, the gel-matrix layer of the present invention is an agarose gel-matrix layer.

In an alternative preferred embodiment, the gel-matrix layer of the present invention is an acrylamide gel-matrix layer.

The wells formed in the gel-matrix layer can extend through the gel layer so that the bottom surface of the well is formed by the plate on which the gel-matrix layer is formed or may only partially extend through the layer so that the bottom of the well is formed by part of the gel-matrix layer. Preferably, the well is of a size sufficient to contain the sample. Preferably, the wells have a volume of between $3mm^3$ and $25mm^3$, more preferably between $6mm^3$ and $10mm^3$.

The front face of the well is the face of the well through which the sample passes into the gel-matrix layer upon application of an electrophoresis voltage. When the gel-matrix layer is used to separate a negatively charged molecule, such as DNA, the front face of the well is the face closest to the anode.

The front face of the well is curved and/or comprises more than one planar surface. Accordingly, the front face of the well is not a flat planar face. The front face of the well may be any shape provided it is not a flat planar face. Wells incorporating such front faces include wells that in plan view (i.e. viewed from above) appear as triangles, squares, pentagons, hexagons, circles, ovals etc. For example, the front face of the well may be curved, or may comprise the corner of a triangle and thereby comprise two planar faces.

Preferably, the front face of one or more wells of the gel-matrix layer of the present invention is curved. The curved front face may be convex or concave. Furthermore, the front face may comprise a number of curves. It is further preferred that one or more wells of the gel-matrix layer is circular or oval shaped in plan view.

Alternatively, it is preferred that the front face of one or more wells of the gel-matrix layer of the present invention comprises more than one planar surface. Preferably, the front face of the well appears in plan view to comprise one or more corners arising from the intersection of two or more planar surfaces. Preferably, the well is triangular, square, pentagonal or hexagonally shaped in plan view.

By using wells which do not have a planar front face, electrophoresis separates molecules in the samples into bands in the gel-matrix layer which are not linear bands.

Instead, curved, v-shaped etc. bands are formed depending on the shape of the front face of the well. When the front face of the well comprises a number of curves or a number of intersecting planar surfaces, a dotted band may be formed.

As non-linear bands of the molecules in the sample are produced in the gel-matrix layer, this gives at least two major advantages over the prior art methods whereby only linear bands are obtained.

The first advantage is that as the amount of sample in the well varies between the cathode and anode across the width of the well, a dilution effect is seen. For example, a well which has an equilateral triangle shape in plan view, wherein one corner of the triangle faces the anode, will give rise to bands which have their highest sample loading in the centre of the band with a dilution effect towards the edges of the band. The dilution effect ensures that overloaded bands (possibly containing closely running doublets, etc.) which might not have been resolved in the region of heavy loading, will have a much greater chance of being resolved where the loading is effectively diluted between cathode and anode, namely at the edges of the band. Thus, rather than having to run a series of dilutions of samples, often a single well loading could be sufficient. Additionally, some semi-quantification of band content is feasible by examining the taper of signal towards the edges. For example, band intensity can be measured over a narrow segment of the band (segment defined at a particular point in the shape or taper) and compared with the intensity of known standards. The use of phosphoimagers and other imaging techniques to analyse the intensity of bands is well known to those skilled in the art. Furthermore, by determining any change in the length of the well in the line of electrophoresis across the width of the well, the dilution effect can be calculated. For example, if the length of the well at its longest point is 2mm and the length of the well at its shortest point is 0.4mm, then a 1 in 5 fold dilution of the band is obtained across the band.

As different shapes can be used to give different shaped bands, the electrophoresis gel-matrix layer of the present invention can be used in simplifying the direct identification of any bands as belonging to a given well. This will be convenient in high density arrays, where care must be taken to identify which track in the array is which. In high density arrays one could work with overlapping tracks which run into each other. Again this would be simplified by using the shape and content of the band as part of the process of deconvoluting the high density data.

Preferably, the electrophoresis gel-matrix layer of the present invention comprises wells of at least two different shapes formed in the gel-matrix layer. The different shaped wells can be used to help identify which bands belong to which well.

In a preferred embodiment, the gel-matrix layer of the present invention comprises a two-dimensional array of wells as described in WO 96/18891 provided at least one of the wells has a front face which is curved and/or comprises more than one planar surface. It is particularly preferred that the gel-matrix layer of the present invention has a series of wells as shown in Figure 1.

The present invention also provides a method for making the electrophoresis gel-matrix layer of the present invention comprising the steps of forming a gel-matrix layer in a mould, inserting a well-former into the gel-matrix layer before it sets, allowing the gel-matrix layer to set, and removing the well-former.

The method can be used to form agarose or acrylamide gel-matrix layers using suitable moulds well known to those skilled in the art. Furthermore, suitable methods for forming agarose and acrylamide gel-matrix layers having a two dimensional array of wells are described in International patent application WO 96/18891, which is incorporated herein by reference.

The present invention also provides the use of the gel-matrix layer of the present invention in the electrophoresis of a sample. The method preferably comprises the steps of placing the sample into a well of the gel-matrix layer, and subjecting the gel-matrix layer to an electrophoresis voltage so that molecules in the sample passes through the front wall of the well into the gel-matrix layer. Preferably, the sample contains DNA or RNA molecules.

The present invention also relates to a kit comprising a mould for forming a gel-matrix layer and a well-former, wherein the well-former forms one or more wells having a front face which is curved and/or comprises more than one planar surface. Preferably, the well-former and the mould are adapted to interact so that when the well-former interacts with the mould it is fixed in position while the gel sets.

The present invention also relates to a well-former which is adapted to removably interact with a mould for forming a gel-matrix layer, wherein when the well-former interacts with the mould it is fixed in position so that one or more wells having a front face which is curved and/or comprises more than one planar surface are formed in the gel-matrix layer.

The present invention is now described further, by way of example only, with reference to the accompanying Figures in which:

Figure 1 is a plan view of wells of various shapes encompassed by the present invention formed in an electrophoresis gel-matrix layer using a two-dimensional well-former.

Figure 2 shows the result of the performance of electrophoresis on a gel-matrix layer wherein the bands of DNA can clearly be seen to be v-shaped.

Example 1

Figure 1 shows a plan view of wells formed in an electrophoresis gel-matrix agarose layer (1). The wells have been formed in a two-dimensional array in accordance with the method disclosed in International patent application WO 96/18891. The wells (2) are arranged in a square lattice pattern with a 9mm centre-to-centre spacing. The wells (2) are arranged in 12 rows of 8 in accordance with that of a standard microtitre plate. Each row of wells (2) has a different shape enabling easier identification of bands derived from a particular well (2).

Example 2

A 7.5% polyacrylamide gel in 1 x tris-borate-EDTA (TBE) buffer was prepared in accordance with standard procedures. Wells were formed in the gel by using square pegs rotated through 45° so that a corner of the square faces the anode during electrophoresis. Accordingly, the front face of the wells comprises two planar faces of the well.

A polymorphic PCR amplicon was loaded into each well and an electrophoretic voltage applied across the gel leading to the migration of the DNA into the gel.

The gel was removed from the electrophoresis apparatus and the band visualised using ethidium bromide. A figure of the gel obtained is shown in Figure 2. The bands of DNA (3) can clearly be seen in the gel as well as the dilution effect of using a well with a non-planar front face. In particular, it can be seen at the lateral corners of the v-shaped band that the signal is fainter and that therefore such non-linear shaped bands can be advantageously used when reading over-loaded samples and also for gauging approximate sample concentration.

It will of course be understood that the present invention has been described purely by way of example, and that modifications of details can be made within the scope of the invention as defined in the accompanying claims.

CLAIMS

1. An electrophoresis gel-matrix layer having one or more wells formed in the gel-matrix layer, wherein the front face of the well is curved and/or comprises more than one planar surface.

2. The electrophoresis gel-matrix layer of claim 1, wherein the gel-matrix layer is agarose gel-matrix layer.

3. The electrophoresis gel-matrix layer of claim 1, wherein the gel-matrix layer is acrylamide gel-matrix layer.

4. The electrophoresis gel-matrix layer of any one of the previous claims, wherein the one or more wells have a volume of between $3mm^3$ and $25mm^3$.

5. The electrophoresis gel-matrix layer of any one of the previous claims, wherein the one or more wells appear as triangles, squares, pentagons, hexagons, circles or ovals in plan view.

6. The electrophoresis gel-matrix layer of any one of the previous claims, wherein the gel-matrix layer comprises a two-dimensional array of wells.

7. The electrophoresis gel-matrix layer of any one of the previous claims comprising wells of at least two different shapes formed in the gel-matrix layer.

8. A method for making the electrophoresis gel-matrix layer of any one of claims 1 to 7 comprising the steps of forming a gel-matrix layer in a mould, inserting a well-former into the gel-matrix layer before it sets, allowing the gel-matrix layer to set, and removing the well-former.

9. Use of the gel-matrix layer of any one of claims 1 to 7 in the electrophoresis of a sample.

10. The use of claim 9 comprising the steps of placing the sample into a well of the gel-matrix layer, and subjecting the gel-matrix layer to an electrophoresis voltage so that molecules in the sample passes through the front wall of the well into the gel-matrix layer.

11. The use of claim 10, wherein the molecules in the sample are DNA or RNA molecules.

12. A kit for making the gel-matrix layer of any one of claims 1 to 7 comprising a mould for forming a gel-matrix layer and a well-former, wherein the well-former forms one or more wells having a front face which is curved and/or comprises more than one planar surface.

13. A well-former which is adapted to removably interact with a mould for forming a gel-matrix layer, wherein when the well-former interacts with the mould it is fixed in position so that one or more wells having a front face which is curved and/or comprises more than one planar surface are formed in the gel-matrix layer.

FIG. 1

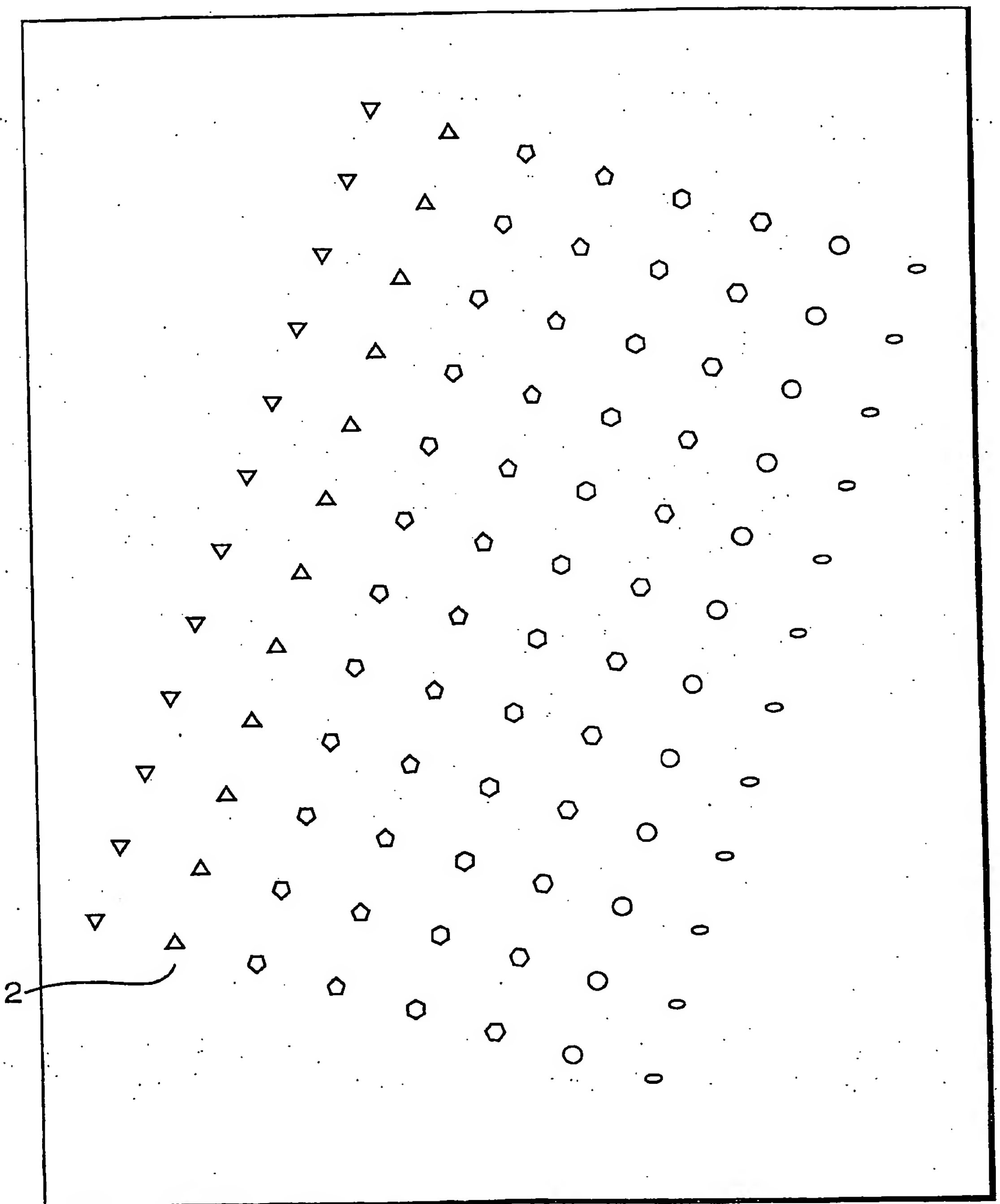
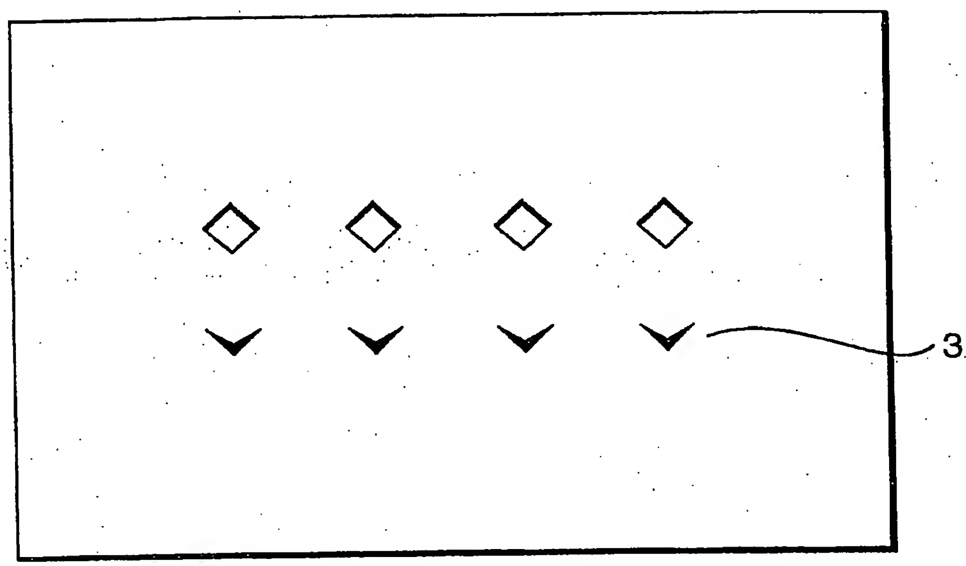


FIG. 2



INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03053

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N27/447

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 074 981 A (FAIRFIELD FREDERIC R) 24 December 1991 (1991-12-24) column 11, line 16 - line 41; figure 12	1,8,9, 12,13
Y	WO 96 18891 A (UNIV LONDON ;DAY IAN NICHOLAS MONSARRATT (GB)) 20 June 1996 (1996-06-20) cited in the application the whole document	1,8,9, 12,13
A	US 5 324 412 A (KOLNER DOUGLAS E) 28 June 1994 (1994-06-28) column 3, line 41 - line 44; figure 1	1
A	US 5 073 246 A (BARICH JOHN J ET AL) 17 December 1991 (1991-12-17) cited in the application the whole document	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 January 2000	13/01/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Duchatellier, M

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/03053

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
US 5074981	A	24-12-1991	NONE			
WO 9618891	A	20-06-1996	JP	10510624	T	13-10-1998
US 5324412	A	28-06-1994	NONE			
US 5073246	A	17-12-1991	CA	2042641	A	17-11-1991
			DE	4114348	A	21-11-1991
			FR	2662097	A	22-11-1991
			GB	2245069	A,B	18-12-1991
			GB	2274715	A,B	03-08-1994
			JP	2020488	C	19-02-1996
			JP	4231490	A	20-08-1992
			JP	7057916	B	21-06-1995